

REMARKS

Status of the Claims

Claims 1-25 are pending in the application. Claims 21-23 have been withdrawn as drawn to a non-elected invention. Claims 1, 3, 5, 10-14, and 16 have been amended.

Claim Amendments

Claim 1 has been amended to clarify the steps of the claim and to recite that the method comprises providing an assay system comprising MAPK polypeptide comprising SEQ ID NO: 36, 37, 38 and 40 or MAPK nucleic acid comprising SEQ ID NO: 1, 10, 12, 13, 21, and 29, wherein the assay system is capable of detecting the activity or expression of MAPK; contacting the assay system with a test agent that modulates the activity or expression of MAPK; and determining the activity or expression of the MAPK polypeptide or nucleic acid in the assay system in the presence or absence of the test agent of step (b), wherein a change in MAPK activity or expression between the presence and absence of the test agent identifies the test agent as a candidate RAC, axin, and beta-catenin pathways modulating agent. Support for the amendment is found throughout the application and in particular can be found at, for example, pages 6-7.

Claims 3, 5, and 12-15 have been amended to provide minor clarifying amendments and to correct matters of form.

Claim 16 has been amended in step (d) to clarify that the second assay is capable of detecting change in the RAC, axin, and beta-catenin pathways, in step (e) to recite that the second assay system is contacted with the test agent of step (b), and in step (f) to recite that the Rac, axin, and beta-catenin pathways are measured in the presence or absence of the test agent, wherein the detection of a difference in the presence and absence of the test agent confirms the test agent as a RAC, axin, and beta-catenin pathways modulating agent. Support for the amendment is found throughout the application.

The claim amendments are made solely in an effort to advance prosecution and are made without prejudice, without intent to acquiesce in any rejection of record, and without intent to abandon any previously claimed subject matter. No new matter has been added by way of these amendments.

35 U.S.C. § 112, First Paragraph, Rejections

Enablement

Claims 1-22 were rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the enablement requirement because the claim(s) contains subject matter that was not described in the specification in such a way so as to enable one skilled in the art to which it pertains, or with which it is most closely connected, to make and/or use the invention. Applicants respectfully traverse the rejections.

The Office acknowledged the genetic screen described in the instant specification, but asserted that claimed invention lacks enablement because (1) the claimed assays are based on an extrapolation that SEQ ID NOs: 36, 37, and 38 share 45%, 48%, and 45% amino acid identity with ZC504.4 and therefore in the absence of identified conserved region(s) of interaction between RAC, axin, and beta-catenin and MAPK, one of ordinary skill in the art would not jump to the conclusion that any compound capable of modulating ZC504.4 is definitely involved in the Rac, axin, and beta-catenin pathways since more experiments are need to verify this conclusion; and (2) some compounds (capable of modulating ZC504.4) which fall outside the homology range (i.e, not homologous with MAPK) will be falsely identified as potential modifiers. The Office concluded that the “insufficient disclosure” relating to ZC504.4 would impose undue experimentation to further verify whether the identified compounds are in fact associated with the Rac, axin, and beta-catenin pathways.

The test of enablement is whether one reasonably skilled in the art (1) could make and use the invention (2) from the disclosures in the application coupled with information known in the art (3) without undue experimentation. *In*

re Wands, 858 F.2d 731 (Fed. Cir. 1988); *United States v. Teletronics, Inc.*, 857 F.2d 778 (Fed. Cir. 1988); M.P.E.P. § 2164.01. Thus, under 35 U. S. C. § 112, all that is required is that the specification describe the invention in such terms as to enable a person skilled in the art to make and use the invention.

Contrary to the Office's allegation, the specification clearly teaches one skilled in the art how to make and use the claimed assay for identifying a candidate Rac, axin, and beta-catenin pathways modulating agent. First, the specification describes the function and structure of the MAPK polypeptide and further provides several MAPK polypeptide and nucleic acid sequences that can be used in the screening assays at pages 6-10. In addition, the specification clearly provides numerous examples of assays using the described MAPK polypeptides and nucleic acids that can be employed to identify a candidate Rac, axin, and beta-catenin pathways modulating agent. (Specification at pages 22-32). Furthermore, the specification provides numerous examples of assays that can be used to confirm that the identified agent is a RAC pathway modulating agent. (Specification at 32-35 and 40-450. Applicant submits that performing the assays described in the specification is within the skill of the ordinary artisan.

The Office alleged that the claimed assays are not enabled because there is no data linking MAPK with the Rac, axin, and beta-catenin pathways and therefore further investigation is required to verify whether MAPK is involved in the Rac, axin, and beta-catenin pathways. Contrary to the Office's contention, the specification provides data linking MAPK with the Rac, axin, and beta-catenin pathways. With respect to the Rac pathway, the specification teaches that *ced-10*, *mig-2*, and *rac-2* encode RAC-related proteins and that these genes function to control a number of cell and axonal migrations in *C.elegans*. Inactivation of two or three of these genes causes significant migration defects, whereas mutation in only one of these genes does not. Thus, *ced-10/mig-2* double mutants have gross morphological and movement defects not seen in either single mutant. Specifically, the phenotype of the *ced-10/mig-2* double mutant includes slow growth, vulval withered tail, and sterility defects, none of which is seen with either single mutant (specification at pages 1 and 37). Thus, *ced-10*

and *mig-2* single mutants resemble wildtype worms in morphology and movement, whereas *ced-10/mig-2* double mutants have strong morphological and movement defects.

The link between MAPK and the Rac pathway was determined using two separate assays involving *C.elegans* in which a specific gene was inactivated by RNAi. The assay methods and results are described in the specification at pages 5-6 and 37. Herein, the Applicants describe a first assay in which wildtype *C.elegans*, single *ced-10* mutants, and single *mig-2* mutants, each having the same specific gene inactivated by RNAi were observed for morphological and movement defects resembling those of the *ced-10/mig-2* double mutants.

Those genes that, when inactivated, result in a worm with a double *ced-10/mig-2* mutant phenotype in the single *ced-10* or single *mig-2* mutant *C.elegans* and not in the wildtype *C.elegans* were furthered studied in a second direct cell migration assay. The direct cell migration assay measures the migration of a subset of mechanosensory neurons (AVM and ALM) in *C.elegans* larvae. Those larvae having the *ced-10/mig-2* double mutation show short or misguided AVM and ALM migration compared to wildtype larvae or larvae having the single *ced-10* or single *mig-2* mutation.

The migration of AVM and ALM cells in worms subjected to RNAi treatment demonstrating a double *ced-10/mig-2* mutant phenotype in single *ced-10* or single *mig-2* mutant *C.elegans* was compared with the migration of AVM and ALM cells in (1) wildtype *C.elegans*, (2) *C.elegans* single *ced-10* mutants, and (3) *C.elegans* single *mig-2* mutants. Those genes that, when inactivated by RNAi treatment, cause short or misguided migration of AVM and ALM cells (as compared with wildtype and single mutant *C.elegans*) are relevant to the Rac pathway. One such gene, ZC504.4, was identified. In other words, inactivation of ZC504.4 causes short or misguided cell migration in *C.elegans*.

With respect to the axin pathway, the specification teaches at pages 5-6 and 37-38 that *pry-1* mutant mu38 worms grown at 15°C produces a ruptured vulva (*Rvl*/ axin) phenotype, by which about 95% of animals because eviscerated and die at the L4 molt. Inactivation of the beta-catenin ortholog *bar-1* and the

TCF ortholog *pop-1* suppresses the *pry-1* Rvl/axin mutant phenotype. The link between MAPK and the axin pathway was determined using an assay involving *C.elegans* in which a specific gene was inactivated by RNAi in *pry-1(mu38)* L1 larvae and suppression of the *Rvl* phenotype was scored as a significantly significant increase in the proportion of larvae that survived to adulthood without rupturing. Those genes that, when inactivated, result in a worm with a suppressed *pry-1(mu38)* phenotype in *pry-1(mu38)* L1 larvae, but not in wildtype or unrelated mutants (*lin-1/Ets* and *daf-18/PTEN*, which are unrelated to beta-catenin) were considered specific *pry1/axin* suppressors. The ZC504.4 gene was identified in the screen. Thus, inactivation of ZC504.4 suppresses the *Rvl/axin* phenotype in *C.elegans*.

With respect to the beta-catenin pathway, the specification teaches at pages 5-6 and 38-39 that *hmp-2* mutant worms have a defective body elongation phenotype due to a cell adhesion defect associated with the beta-catenin pathway, which causes *hmp-2* (qm39) mutants to have a lumpy appearance when grown at 15°C. Inactivation of the beta-catenin ortholog *bar-1* and the TCF ortholog *pop-1* suppresses the *pry-1* Rvl/axin mutant phenotype. The link between MAPK and the axin pathway was determined using an assay involving *C.elegans* in which a specific gene was inactivated by dsRNA in *hmp-2(qm39)* L4 larvae which were allowed to lay progeny. Suppression of the *hmp-2(qm39)* phenotype was scored for a modification of the lumpy phenotype, with an increase in dead embryos scored as an enhancement and an increase in wildtype embryos as a suppression of the defective phenotype. The ZC504.4 gene was identified as a suppressor. In other words, inactivation of ZC504.4 suppresses the *hmp-2/beta-catenin* phenotype in *C.elegans*.

Applicants submit that the functional role of ZC504.4 as a modulator of the Rac, axin, and beta-catenin pathways is clearly laid out in the specification. *Ced-10* and *mig-2* are two worm genes that are well established as worm counterparts of Rac pathway genes. Likewise, *pry-1* and *hmp-2* are worm genes that are well-established as counterparts of axin and beta-catenin pathway genes, respectively. As discussed above, previous genetic studies have

demonstrated that animals with defects in the describe genes show certain characteristic phenotypic defects which are indicative of loss of function of the Rac, axin, and beta-catenin pathways in the worm. The RNAi based screens described in Example I of the specification demonstrates that RNAi of the ZC504.4 gene in either a *ced-10* or *mig-2* single mutant worms, but not wildtype worms, recapitulates both of the two phenotypes that are characteristic of defects in the worm Rac pathway: (i) the gross morphological and movement defects and (ii) the specific ALM and AVM neuron migration defects. Likewise, the RNAi based screens described in Example II demonstrates that RNAi of the ZC504.4 gene in *pry-1* mutant worms, but not wildtype or unrelated mutant worms, suppresses the *pry-1*/axon phenotype (ruptured vulva) characteristic of defects in the worm axin pathway. Similarly, the dsRNA screen described in Example III demonstrates that RNAi of the ZC504.4 gene in *hmp-2* mutant worms suppresses the *hmp-2*/beta-catenin phenotype (lumpy larvae) characteristic of defects in the worm beta-catenin pathway.

The different, independent genetic analyses reported in the specification demonstrate unambiguously that worm gene ZC504.4 has a normal function that is tied to modulation of the *ced-10* and *mig-2* genes, the *pry-1* gene and the *hmp-2* gene, and therefore ZC504.4 clearly regulates the worm Rac, axin and beta-catenin pathways. For example, the RNAi results show that agents that block ZC504.4 function in the worm (in this case an inhibitory RNA homologous to ZC504.4) suppressed defects caused by mutations in the worm beta-catenin homolog *hmp-2*. Human cancer cells often carry mutations in beta-catenin genes that contribute to the oncogenic behavior of these cells. The worm beta-catenin pathway genes have human counterparts and therefore it can be extrapolated from the genetic results reported in the specification that inhibition of the human counterparts of worm ZC504.4 will similarly suppress defects in beta-catenin pathway function that contribute to oncogenic behavior of human tumors. Also, the RNAi results show that agents that block ZC504.4 function in the worm suppressed defects caused by mutations in the worm axin homolog *pry-1*. Decreased axin expression in human tumor cells has been correlated with

increased oncogenic properties. Accordingly, as the worm axin pathway genes have human counterparts it can be extrapolated from the genetic results reported in the specification that inhibition of the human counterparts of worm ZC504.4 will similarly suppress defects in axin function that contribute to oncogenic behavior. Lastly, the RNAi results show that agents that block ZC504.4 enhanced the effects of mutations in worm Rac homolog genes *ced-10* and *mig-2*, and thereby showing that ZC504.4 has a normal function in stimulating activity of the worm Rac pathway. Rac/Rho protein overexpression has been found in a large variety of human tumors, and apparently contributes to the oncogenic properties human tumors. Consequently, as the worm Rac pathway genes have human counterparts it can be extrapolated from the genetic results reported in the specification that inhibition of the human counterparts of ZC504.4 will reduce Rac pathway activity and oncogenic properties.

One skilled in the art of genetic screening would understand that the screening assays used and described in the specification are evidence of a link between MAPK and the Rac, axin, and beta-catenin pathways. Specifically, Applicants have shown that inactivation of ZC504.4/MAPK by RNAi results in Rac-associated (i.e., *ced-10* or *mig-2* associated), axin-associated (*pry-1* associated), and beta-catenin-associated (*hmp-2* associated) changes in cell migration and cell adhesion. Therefore, agents that modulate MAPK (inhibit or enhance MAPK) can be used to identify candidate Rac, axin, and beta-catenin pathways modulating agents.

Furthermore, the specification also provides MAPK expression data and MAPK functional data, which further supports the involvement of MAPK in the Rac, axin, and beta-catenin pathways. It is known by ordinary skilled artisans that many tumor cells exhibit altered (typically enhanced) cell migration and cell adhesion characteristics. As further evidence of MAPK's role in a disease associated with altered cell adhesion and migration, Applicants have shown that various tumor cells have overexpressed MAPK compared to tissue-matched normal cells. (specification at pages 43-44). In addition, the specification provides the results of several functional assays, which show that RNAi against

MAPK leads to decreased cell proliferation and growth, and increased apoptosis in various cancer cells. Other studies show that overexpression of MAPK results in increased cell colony growth and an increased expression of certain transcription factors. (specification at pages 44-45).

Based on these teachings and the experimental results of the genetic screens, expression studies, and functional assay studies, Applicants submit that the specification clearly teaches one skilled in the art how to make and use the claimed assay for identifying a candidate Rac, axin, and beta-catenin pathways modulating agent.

The Office contended that the claimed method lacks enablement because the claimed assays are based on an extrapolation that SEQ ID NOs: 36, 37, and 38 share 45%, 48%, and 45% amino acid identity with ZC504.4 and therefore one would not "jump to the conclusion" that any compound capable of modulating ZC504.4 is definitely involved in the Rac, axin, and beta-catenin pathways. Applicants respectfully disagree. The specification reports that human MAPK was identified as the "ortholog" of worm ZC504.4 using blast analysis (the accepted criteria for orthology). Thus, MAPK was identified as the human counterpart of the worm ZC504.4 gene, not because of an arbitrary percentage of sequence homology, but because the blast results demonstrated that of all human proteins human MAPK protein has the greatest percent homology to the worm ZC504.4 protein, and conversely, of all worm proteins, worm ZC504.4 protein has the greatest percent homology to human MAPK protein. Moreover, in comparing human MAPK and *C. elegans* ZC504.4 proteins, the 45-48% identity over the entire length of the protein is not only significant but extraordinary given the length over which the homologies are calculated and the great evolutionary distance between worms and humans (over 600 million years), and would lead a person skilled in the art to conclude that it can only reflect functional conservation. Applicants respectfully point out that no evidentiary basis has been provided by the Office for the assertion that 45-48% identity is not high enough to clarify functional conservation between a worm and human protein, which assertion Applicants submit is unfounded and contrary to

scientifically-accepted teachings. For example, Doolittle, R.F., 1987, *OF URFS AND ORFS, A Primer on How to Analyze Derived Amino Acid Sequences*, University Science Books, Mill Valley, California, presents the homology criteria accepted in the art (chapter attached). The most relevant text is on page 12 where the author states under "Significance: Some Rules of Thumb":

"At this point, let me offer some "rules of thumb" about degrees of confidence. If two sequences are longer than 100 residues, and are more than 25% identical after suitable gapping, it is very likely that the sequences are related". P. 12.

The Office also asserted that some compounds (capable of modulating ZC504.4) which fall outside the homology range (i.e, not homologous with MAPK) will be falsely identified as potential modifiers. Applicants submit that they have clearly demonstrated that ZC504.4 is specifically involved in the Rac, axin, and beta-catenin pathways (its absence did not affect control cells or cells mutated in unrelated cellular pathways) and have further confirmed the involvement of MAPK in the Rac, axin, and beta-catenin pathways via its substantial homology to ZC504.4 and the expression and functional assays studies.

The Office finally concluded that the "insufficient disclosure" relating to ZC504.4 would impose undue experimentation to further verify whether the identified compounds are in fact associated with the Rac, axin, and beta-catenin pathways. Applicants disagree with the Office's contention. The specification provides numerous examples of assays, well within the skill of the ordinary artisan, that can be used for such verification as a matter of routine experimentation. For example, the specification teaches (i) cell adhesion assays, (ii) cell migration/invasion assays, (iii) tubulogenesis assays, and (iv) sprouting assays for verifying modulation of the Rac, axin, and beta-catenin pathways pathway (pages 22-32), as well as angiogenesis, tumorigenicity, and hollow fiber assays (pages 33-34).

Applicants submit that the claimed methods are fully enabled for the reasons set forth above. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection of claims 1-22 under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the enablement requirement.

Written Description

Claims 1-22 were rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the written description requirement because the claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. Applicants respectively traverse the rejections.

The Office asserted that claims do not satisfy the written description requirement because the specification only discloses one species, ZC504.4, of the MAPK genus and therefore did not show possession of the entire genus. Without acceding to the merits of the rejection and solely to advance prosecution, Applicants have amended the claims to recite the sequences of the specified MAPK polypeptides (SEQ ID NOs: 36, 37, 38 and 40) and MAPK nucleic acids (SEQ ID NOs: 1, 10, 12, 13, 21, and 29) to be used in the screening assays, which structure (sequence) is fully known and described in accordance with the written description requirement.

For the reasons indicated above, Applicants submit that the specification demonstrates possession of the claimed invention and thereby satisfies the written description requirement. Accordingly, Applicants respectfully request reconsideration and withdrawal of the rejection of claims 1-22 under 35 U.S.C. § 112, first paragraph for allegedly failing to comply with the written description requirement.

CONCLUSION

In view of the foregoing, the applicants respectfully request reconsideration of the pending claims. If it is believed that such contact would expedite prosecution of the present patent application, the Patent Office is urged to contact the undersigned.

Respectfully submitted,

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